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Inflammation marker and risk of pancreatic cancer: a nested case-control study within the EPIC cohort

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Abstract: BACKGROUND: Established risk factors for pancreatic cancer include smoking, long-standing diabetes, high body fatness, and chronic pancreatitis, all of which can be characterised by aspects of inflammatory processes. However, prospective studies investigating the relation between inflammatory markers and pancreatic cancer risk are scarce. METHODS: We conducted a nested case-control study within the European Prospective Investigation into Cancer and Nutrition, measuring prediagnostic blood levels of C-reactive protein (CRP), interleukin-6 (IL-6), and soluble receptors of tumour necrosis factor- (sTNF-R1, R2) in 455 pancreatic cancer cases and 455 matched controls. Odds ratios (ORs) were estimated using conditional logistic regression models. RESULTS: None of the inflammatory markers were significantly associated with risk of pancreatic cancer overall, although a borderline significant association was observed for higher circulating sTNF-R2 (crude OR=1.52 (95% confidence interval (CI) 0.97-2.39), highest vs lowest quartile). In women, however, higher sTNF-R1 levels were significantly associated with risk of pancreatic cancer (crude OR=1.97 (95% CI 1.02-3.79)). For sTNF-R2, risk associations seemed to be stronger for diabetic individuals and those with a higher BMI. CONCLUSION: Prospectively, CRP and IL-6 do not seem to have a role in our study with respect to risk of pancreatic cancer, whereas sTNF-R1 seemed to be a risk factor in women and sTNF-R2 might be a mediator in the risk relationship between overweight and diabetes with pancreatic cancer. Further large prospective studies are needed to clarify the role of proinflammatory proteins and cytokines in the pathogenesis of exocrine pancreatic cancer.

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Inflammation marker and risk of pancreatic cancer: a nested case-control study within the EPIC cohort

Running title: Inflammation and pancreatic cancer risk

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Abstract (247 words)

Background: Established risk factors for pancreatic cancer include smoking, long-standing diabetes, high body fatness, and chronic pancreatitis, all of which can be characterized by aspects of inflammatory processes. However, prospective studies investigating the relation between inflammatory markers and pancreatic cancer risk are scarce.

Methods: We conducted a nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC), measuring pre-diagnostic blood levels of C-reactive protein (CRP), interleukin 6 (IL-6) and soluble receptors of tumour necrosis factor α (sTNF-R1, R2) in 455 pancreatic cancer cases and 455 matched controls. Odds ratios (OR) were estimated using conditional logistic regression models.

Results: None of the inflammatory markers were significantly associated with risk of pancreatic cancer overall, although a borderline significant association was observed for higher circulating sTNF-R2 (crude OR = 1.52 [95% CI 0.97 – 2.39], highest vs. lowest quartile). In women, however, higher sTNF-R1 levels were significantly associated with risk of pancreatic cancer (crude OR = 1.97 [95% CI 1.02-3.79]). For sTNF-R2, risk associations seemed to be stronger for diabetic individuals and those with a higher BMI.

Conclusion: Prospectively, CRP and IL-6 do not seem to play a role in our study with respect to risk of pancreatic cancer, whereas sTNF-R1 seemed to be a risk factor in women and sTNF-R2 might be a mediator in the risk relationship between overweight and diabetes with pancreatic cancer. Further large prospective studies are needed to clarify the role of pro-inflammatory proteins and cytokines in the pathogenesis of exocrine pancreatic cancer..

Key words: inflammation, pancreatic cancer, EPIC, CRP, IL-6, TNF receptor

Introduction

Evidence is accumulating that systemic low-grade chronic inflammation in addition to local inflammation in the pancreas is involved in the pathogenesis of pancreatic cancer (Farrow & Evers, 2002; McKay *et al*, 2008; Whitcomb, 2004). Research findings pointing to this direction include the documented relationship of pancreatic cancer risk with chronic pancreatitis (Raimondi *et al*, 2010), as well as with smoking (Lynch *et al*, 2009; Vrieling *et al*, 2010), pre-existing and long-standing diabetes mellitus (Huxley *et al*, 2005), and excess weight (Genkinger *et al*, 2010), all of which are known or suggestive determinants of low-grade inflammatory states (Goncalves *et al*, 2011; Hotamisligil, 2006; Kolb & Mandrup-Poulsen, 2005; Whitcomb, 2004).

Even though the mechanisms by which chronic inflammation leads to carcinogenesis are not fully understood, it is generally accepted that inflammation results in repeated DNA damage and in the accumulation of genetic defects (McKay *et al*, 2008). However, pro-inflammatory cytokines and growth factors are also released in response to the tumour, making it difficult to distinguish between cause and effect in the inflammatory processes (McKay *et al*, 2008).

Circulating C-reactive protein (CRP) concentration, an acute-phase protein produced in the liver, is increased in pancreatic cancer patients (Barber *et al*, 1999; Moses *et al*, 2009; Mroczko *et al*, 2010), most likely as part of the systemic inflammatory response to the tumour. Interleukin-6 (IL-6) and tumour necrosis factor alpha (TNF-α) are up-regulating factors of CRP and have also been shown to be increased in pancreatic cancer patients (Barber *et al*, 1999; Ebrahimi *et al*, 2004; Moses *et al*, 2009; Mroczko *et al*, 2010; Talar-Wojnarowska *et al*, 2009). Prospectively, increased levels of CRP have inconsistently been associated with pancreatic cancer risk. To our knowledge, prospective studies on the association of IL-6, TNF-α, or its receptors with risk of pancreatic cancer are lacking.

We measured prediagnostic concentrations of CRP, IL-6, and soluble TNF receptors (sTNF-R1 and R2) in blood samples of 455 primary exocrine pancreatic cancer cases and 455 individually matched controls within the Prospective Investigation into Cancer and Nutrition (EPIC) as possible reflections of either pancreatic cancer or a metabolic risk factor potentially increasing pancreatic cancer risk by aggravating pancreatic inflammatory disease.

Materials and Methods

Study Population

The European Prospective Investigation into Cancer and Nutrition (EPIC) is a large cohort study conducted in 23 centres in ten European countries (Denmark, France, Germany, Greece, Italy, the Netherlands, Spain, Sweden, and the United Kingdom). Detailed descriptions of study design, population, and baseline data collection of the cohort can be found elsewhere (Haftenberger *et al*, 2002; Riboli *et al*, 2002). Briefly, about 370 000 women and 150 000 men were enrolled between 1992 and 2000. Participants provided information on dietary habits and lifestyle factors, and in addition, weight, height, and waist and hip circumferences were measured at baseline. Each participant provided informed consent, and the local ethical review committees approved the EPIC cohort study as well as the current project.

Blood Sample Collection and Storage

In the seven EPIC core countries (France, Germany, Greece, Italy, the Netherlands, Spain, and the United Kingdom), blood samples were collected at baseline, based on a standardized protocol and aliquoted in plastic straws (plasma, serum, erythrocytes, and buffy coat for DNA). The aliquoted specimens were then stored in a central biorepository in liquid nitrogen (-196°C). In Sweden, all samples were stored locally in freezers at -70°C and in Denmark in nitrogen vapour (-150°C). In the present study, Norway was excluded because blood samples were only recently collected and very few pancreatic cancer cases have been diagnosed after blood donation.

Follow-up for Cancer Incidence and Vital Status

In six of the participating countries (Denmark, Italy, the Netherlands, Spain, Sweden, and the United Kingdom), follow-up of cancer cases was based on population registries. In the other three countries (France, Germany, and Greece), a combination of methods was used including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. In all EPIC centres, data on vital status is collected from mortality registries at the regional or national level, which is combined with health insurance data (France) or data collected by active follow-up (Greece). Cases reported in this study were all diagnosed up to the latest dates of complete follow-up, which was between December 2002 and 2005, depending on the study centre. For Germany, Greece, and France, the end of follow-up was the last known contact, date of diagnosis, or date of death, whichever came first.

Selection of Case and Control Subjects

Up to December 2006, follow-up has led to the identification of 578 incident cases of non-endocrine pancreas cancer that were coded according to ICD-10 (C25.0-25.3, 25.7-25.9), and for 455 of these cases blood specimens were available. Exclusion criteria were occurrence of other malignant tumours preceding the diagnosis of pancreatic cancer, except for non-melanoma skin cancer. Of the 455 cases, 334 (76%) were microscopically confirmed and the remaining 24% were diagnosed by imaging results, physical examination, or clinical symptoms. Most tumours occurred in the head of the pancreas (42%), followed by body (7%) and tail (5%), while the rest of the tumours were of unknown localization. For each case, one control subject was selected, that was alive and free of cancer at the time the index case was diagnosed, using an incidence density sampling procedure. All identified cases were matched with one control by centre, sex, age at blood collection (+/- 3 years), date of blood donation (+/- 3 months), time of blood donation (+/- 2 hours), fasting status (<3 hours, 3-6 hours, >6 hours after last meal) and use of hormones (oral contraceptive pill, hormone or oestrogen replacement therapy).

Laboratory Assays

Plasma (in Scandinavian samples) and serum concentrations of CRP were measured by multiplex immunoassays using the Fluorokine MAP Obesity Base Kit (R&D Systems, Inc., Minneapolis, MN, USA). IL-6 and sTNF receptors were measured by enzyme linked immune sorbent assays (ELISA) using the Quantikine kit (R&D Systems, Inc., Minneapolis, MN, USA). The total amount of free receptor plus the total amount of receptor bound to TNF is measured with this method. All measurements were performed in our specialized immunoassay laboratory of the Division of Cancer Epidemiology (German Cancer Research Center, Heidelberg, Germany). Samples of cases and matched controls were analyzed within the same analytical batch. Intra-batch and inter-batch coefficients of variation were 6.6 and 10.8% for IL-6, 3.6 and 4.1% for sTNF-R1, 5.5 and 11.0% for sTNF-R2, and 10.3 and 11.6% for CRP. Units of IL-6 are expressed as pg/mL, of sTNF receptors as ng/mL, and of CRP as mg/L. One batch during the sTNF-R2 measurements did not perform well and, therefore, 70 subjects were excluded due to technically invalid results (all from Malmo, Sweden).

Statistical Analysis

Case and control differences across baseline characteristics were assessed by paired t-tests (continuous variables) or by generalized McNemar's Test (categorical variables). Spearman's partial rank correlation coefficients [r] adjusted for age, sex, and

EPIC recruitment centre were used to assess the strength of associations between waist circumference, waist-hip-ratio, BMI, glycated haemoglobin (HbA1c), and inflammatory markers, as well as for the correlation between the inflammatory markers.

Odds ratios (OR) and corresponding 95% confidence intervals (CI) for pancreatic cancer at different serum levels of IL-6, sTNF receptors, and CRP were calculated by conditional logistic regression models, using the exposure assessments of the matched case-control sets. Continuous measurements of the inflammatory markers were log2 transformed to achieve approximate normality. In this scale, a unit increase corresponds to a doubling of concentration. Quartile cut-points were based on the distribution of biomarkers among controls. Sex-specific quartile cut-points had a negligible effect on risk estimates and were, therefore, not applied. Modelling the median within each quartile as a continuous variable was used to assess linear trends in ORs. Testing the model fit for categorical vs. continuous models resulted in very similar AICs, with a slightly better fit for the latter model.

Inflammatory markers may be downstream in the causal chain of excess body weight, smoking, or diabetes and pancreatic cancer. Alternatively, other pathways might explain associations of these conditions with risk of pancreatic cancer and, hence, inflammatory markers may be independently related to cancer or not at all. We tried to elucidate these rather complex and yet unknown relationships in our study by applying different adjustment models and by performing several subgroups analyses. All these models and methods are of exploratory nature in our study.

Potential confounding of factors other than those controlled for by matching were examined by assessing the association of these factors with pancreatic cancer risk using unconditional logistic regression models adjusted for matching factors, by correlation analyses, and by including these as additional factors in conditional logistic regression models. BMI, waist-hip-ratio, waist circumference, alcohol consumption, current and past tobacco smoking, and diabetes were considered as potential confounders. Variables remained in the models, if they were associated with pancreatic cancer, correlated with the inflammatory markers, or changed the β estimate by more than 10%. Based on these conditions, BMI as a continuous variable and smoking as a categorical variable [never smoking, former smoking (quitting smoking < 10 years ago, ≥ 10 years ago), current smoking (< 10, 10 - 20, ≥ 10 cigarettes a day), missing] were considered as confounding factors and remained in the multivariate adjusted model. To assess a possible confounding effect of diabetes on the risk associations, we controlled for diabetes in further exploratory analyses. Subjects were defined as diabetics if they self-reported the condition in the baseline questionnaire at recruitment (n=52) and/or had glycated haemoglobin (HbA1c) levels ≥ 6.5% in the current study (n=93). This percentage is used as a cut-off for diabetes diagnosis (ADA, 2009). HbA1c has been measured previously in the same study population (Grote *et al*, 2011). Physical activity and socio-economic status did not markedly change the risk estimates and were, therefore, not included in the final model.

Subgroup analyses were performed to assess possible effect modifications by sex, diabetes and smoking status, by median age (62 years), waist circumference (96cm for men, 80 for women), waist-hip-ratio (0.95 for men, 0.80 for women), and median BMI (26.2 kg/m^2 for men, 24.6 for women), or by lag-time (time between blood collection and diagnosis of pancreatic cancer, ≤ vs. > 5 years). Cross-product terms were added in logistic regression models and Wald tests were performed to examine whether any apparent heterogeneity of effect was significant. To limit reverse causation bias which could occur when the advanced tumour causes changes in inflammatory marker levels, we performed subgroup analyses with two years of follow-up as a cut-point (≤ vs. > 2 years).

All statistical analyses were conducted using the Statistical Analysis System (SAS) software package, Version 9.2 (SAS Institute Inc., Cary, North Carolina, USA). All statistical tests were two-tailed and significant at the 5%-level.

Results

Baseline characteristics of pancreatic cancer cases and matched control subjects are shown in **Table 1**. Mean age at recruitment into the initial cohort was 58 years and mean age of cases at pancreatic cancer diagnosis was 63 years, resulting in mean follow-up time of 5.3 years for cases (range 0-13). Female pancreatic cancer cases had a significantly higher BMI and waist-circumference than corresponding controls, but no difference in waist-hip-ratio was observed. For men, however, no significant difference for any of the anthropometric measures comparing cases and controls was seen. A higher percentage of cases currently smoked compared to controls (31 vs. 22%). At baseline, cases also reported more often to be diabetic and/or had HbA1c levels ≥ 6.5% compared with controls (14 vs. 8%). However, these results are not mutually adjusted and serve descriptive purposes only.

Among controls, sTNF-R1 and sTNF-R2 showed a high degree of correlation. The correlation of circulating CRP levels with IL-6, sTNF-R1, and sTNF-R2 concentrations was relatively high with Spearman's rank correlation coefficients up to 0.44. BMI, waist circumference, and waist-hip-ratio correlated moderately with CRP and IL-6 and to lesser extent with sTNF-R1 but not with sTNF-R2 concentrations (**Table 2**). Participants with diabetes (self-reported at baseline and/or HbA1c ≥ 6.5%) and those who smoked had higher levels of CRP and IL-6 than non-diabetics (Table 2). Mutual adjustments for smoking categories and/or BMI resulted in unaltered (diabetes) or stronger associations (smoking, data not shown).

The potential confounders or effect modifiers overweight (OR = 1.05 [95% CI 1.01-1.08], per 5 BMI units), smoking (OR = 1.84 [95% CI 1.30-2.60], current vs. never), and diabetes (OR = 1.74 [95% CI 1.12-2.71]) were associated with risk of pancreatic cancer in our study.

Pancreatic cancer risk tended to be increased with higher levels of sTNF-R2 (crude OR = 1.52 [95% CI 0.97 – 2.39] comparing highest with lowest quartiles, p-trend over quartiles = 0.07); but these associations were not significant at the 5%-level, and BMI and smoking adjustments attenuated the risks of pancreatic cancer (**Table 3**). Elevated CRP (crude OR = 1.36 [95% CI 0.92 – 2.01], p-trend = 0.26), IL-6 (OR = 1.30 [95% CI 0.84 – 2.00], p-trend = 0.61), and sTNF-R1 levels (OR = 1.23 [95% CI 0.78-1.94], p-trend = 0.23) showed no significant association with risk of pancreatic cancer. Adjustments for HbA1c levels and mutually for the other inflammatory markers in addition to BMI and smoking categories attenuated risk estimates for elevated levels of inflammatory markers closer to 1.0 (data not shown). Exclusion of subjects with CRP levels above 10 mg/L (as this is more likely an indication for an acute rather than a chronic inflammatory state) had no effect on the association between CRP levels and pancreatic cancer risk (data not shown). Women tended to be at increased pancreatic cancer risks for higher CRP or sTNF receptor levels, and specifically so for sTNF-R1, although risk estimates were inconsistently significant between categorical and continuous analyses and between crude and BMI and smoking adjusted models (Table 3).

Tests for heterogeneity of continuous sTNF receptors, adjusted for matching factors, resulted in statistically significant differences in pancreatic cancer risk by median BMI, diabetes and smoking status, but not by median waist circumference, waist-hip-ratio or median age. Compared to never smokers, risks in former and current smokers were elevated albeit not statistical significant. Diabetics (p interaction = 0.001) and subjects with a BMI above the median (p interaction = 0.04) had a significantly higher risk of pancreatic cancer with elevated levels of sTNF-R2 than non-diabetics or subjects with lower than median BMI, respectively (**Figure 1b**). Adjusting subgroup analyses for BMI, smoking categories, HbA1c levels, and/or mutually for inflammatory markers attenuated the risk estimates to non-significance (data not shown). Interestingly, higher circulating CRP and IL-6 levels tended to be related to increased pancreatic cancer risk in leaner subjects, although ORs and tests for interaction were not statistically significant (**Figures 1c/d**).

Discussion

In our nested case-control study of 455 pancreatic cancer subjects and 455 individually matched controls higher circulating levels of sTNF-R2, but not of sTNF-R1, CRP, and IL-6 levels tended to be positively associated with the risk of pancreatic cancer. Stratification by sex revealed significantly increased pancreatic cancer risks in women for higher sTNF-R1 levels. Positive associations between sTNF-R2 and pancreatic cancer seemed to be likely for diabetic subjects, those with a higher BMI, and possibly also for smokers.

In the acute-phase response to tissues damage, infection, inflammation, or malignant neoplasia, CRP is increasingly produced by hepatocytes, predominantly under control by IL-6. CRP binds to damaged cell membranes or apoptotic cells, forming an aggregate that activates the complement pathway, resulting in the phagocytosis of the damaged cells and in increased pro-inflammatory pathophysiological effects. CRP, therefore, reflects ongoing inflammation and/or tissue damage and functions as a pro-inflammatory mediator. In this context, it may not only be a marker of a disease, but it may also contribute to pathogenesis (Pepys & Hirschfield, 2003). In several small hospital-based case-control studies, CRP levels were significantly higher in pancreatic cancer cases compared to chronic pancreatitis patients or controls (Barber *et al*, 1999; Moses *et al*, 2009; Mroczko *et al*, 2010). In addition, elevated levels of CRP were associated with a poor prognosis in pancreatic cancer patients (McKay *et al*, 2008). Prospectively, no association was observed in a

Greek study with 14 pancreatic cancer cases (Trichopoulos *et al*, 2006), whereas a weak decrease in pancreatic cancer risk with an OR of 0.94 [95% CI 0.89-0.99] was seen among 311 cases in the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohort of male Finish smokers (Douglas *et al*, 2010). The same authors did not find an association in the Ovarian Cancer Screening Trial (PLCO) or in combined analyses of both cohorts. Our results are in line with the prospective Greek and PLCO study showing no association of CRP with risk of pancreatic cancer.

No prospective study has been conducted so far to assess the association of circulating TNF-α, its soluble receptors, or IL-6 levels with risk of pancreatic cancer, both up-regulators of CRP. TNF-α is a pro-inflammatory cytokine produced by many cell types, including cancer cells, upon exogenous noxious stimuli. The effects of TNF-α are mediated mainly by two receptors, TNF-R1 and TNF-R2, which also circulate in soluble forms upon shedding. TNF receptor activation leads to induction of genes involved in inflammation and cell survival, resulting in the activation of nuclear factor-κB (NF-κB). However, if NF-κB activation is inadequate, apoptosis is mediated via accumulation of reactive oxygen species as a late response to TNF-α. This cytokine, thus, is not only involved in maintenance of the immune system, but also in pathological processes such as malignant diseases. The majority of cell types and tissues express both receptor types (Balkwill, 2006) and among colon cancer patients it has been shown that the concentrations of sTNF-Rs correlate with the stage of disease as tumour cells have a greater tendency than non-malignant cells to shed forms of their cell surface proteins (Aderka, 1996). Soluble TNF receptors can serve as TNF antagonists, carrier proteins of TNF, slow release reservoirs for TNF, and stabilizers of TNF-bioactivity. It is not known, however, whether the two soluble receptors have distinct or similar functions (Aderka, 1996), and based on this, we cannot explain, why we observed a potential increase in pancreatic cancer risk for elevated sTNF-R2 but not for sTNF-R1. It might be, however, that sTNF-R2 plays a more prominent role in pancreatic cancer development. This aspect needs to be explored in functional studies. So far, TNF-α and/or the soluble receptors have been assessed in hospital-based case-control studies with pancreatic cancer patients, observing either higher levels of TNF-α/soluble TNF receptors among pancreatic cancer subjects than among controls (healthy volunteers or chronic pancreatitis patients (Barber *et al*, 1999; Talar-Wojnarowska *et al*, 2009)), or no difference in serum levels (Ebrahimi *et al*, 2004). To our knowledge, our nested-case control study within the prospective EPIC cohort study is the first to address the association of sTNF receptors with risk of pancreatic cancer, and we observed a non-significant increase in risk overall, which was more apparent for sTNF-R2 than sTNF-R1, and which was attenuated after adjustments for smoking status, BMI, and HbA1c levels or diabetes status. It is unclear why we found a difference in risk between men and women with elevated risks for increasing levels of sTNF-R1 in women only.

As with TNF-α, pancreatic cancer patients' IL-6 concentrations have shown to be higher than in healthy controls in hospital-based case-control studies (Barber *et al*, 1999; Ebrahimi *et al*, 2004; Moses *et al*, 2009; Mroczko *et al*, 2010). In contrast to these observations, in our prospective study we did not find elevated pre-diagnostic IL-6 concentrations in subjects who became pancreatic cancer cases later in time compared to non-cancer controls at baseline. IL-6 is synthesized by many cell types in response to stimulation from TNF-α and IL-1 and indirectly regulates cell proliferation and apoptosis through its activation of other factors. Therefore, IL-6 plays a role in chronic inflammation, which may enhance cancer development (Hodge *et al*, 2005). However, due to the small number of prospective studies so far investigating the relationship of IL-6 with cancer, a recent published review concluded that it is yet impossible to determine whether IL-6 is causally related to cancer (Heikkilä *et al*, 2008).

It has been shown in a wide range of studies that CRP, IL-6, TNF-α, and TNF receptor levels vary by body weight, with higher levels among overweight or obese compared to normal weight subjects, and with decreasing levels during weight loss (Forsythe *et al*, 2008; Himmerich *et al*, 2006). Furthermore, compared with never smokers, cigarette smokers also have significantly higher levels of CRP and IL-6, and possibly also of TNF receptors (Fernandez-Real *et al*, 2003). Finally, subclinical systemic inflammation has been reported in type 2 diabetes (Kolb & Mandrup-Poulsen, 2005), including elevated levels of the aforementioned and evaluated parameters in our study. In our study, elevated levels of CRP, IL-6 and sTNF-R1 correlated with excess weight and, in addition, higher CRP and IL-6 levels were associated with smoking and diabetes.

Furthermore, overweight, smoking, or diabetic participants at baseline were at increased pancreatic cancer risk. This risk was even stronger if overweight or diabetic participants had elevated levels of sTNF-R2, even though this marker was not correlated with BMI or associated with diabetes in controls. This can be interpreted as sTNF-R2 being a mediator of the relationship between overweight and/or diabetes and pancreatic cancer. A similar scenario is likely for sTNF-R1, but our results do not clearly support this hypothesis (**Figure 1a**). In contrast, stratification by median BMI, diabetes or smoking status resulted in similar weak risk estimates for elevated CRP and IL-6 concentrations. It seems as if, regardless of the presence of a putative pancreatic cancer risk factor (overweight, diabetes, and smoking), these inflammatory markers are not associated with pancreatic cancer risk themselves. In addition, they also do not appear to be in the causal chain between risk factor and cancer.

Some strengths and limitations of our study should be mentioned. Although a single measurement of a biomarker, as assessed in our study, could result in random misclassification, CRP, IL-6, and sTNF receptors have been shown to be reliably measured over time (Clendenen *et al*, 2010; Gu *et al*, 2009). A major strength of our study is that questionnaire data and blood samples were collected prospectively around the same time point, prior to pancreatic cancer diagnosis, which reduces the possibility of reverse causation bias to some extent. In addition, pancreatic cancer risk seemed to be stronger for elevated sTNF receptor levels among subjects with longer follow-up times. A limitation of our study is that information on pancreatic or liver disorders, on inflammatory diseases, or on use of anti-inflammatory drugs was not recorded for most of the EPIC centres; therefore, controlling for these potential confounders was not possible. Consequently, we cannot exclude the possibility that the observed suggestive increased pancreatic cancer risk among individuals with elevated sTNF-R2 levels may partly be due to chronic pancreatitis or impaired liver function, for example. Furthermore, number of subjects in specific subgroups were rather small, thus, we cannot rule out that results obtained from these analyses are chance findings. Further large prospective studies are needed to verify our results in the respective subgroups with sufficient power to detect significant risk associations.

Conclusion

Prospectively, CRP and IL-6 do so seem to play a role in our study with respect to risk of pancreatic cancer, whereas sTNF-R1 seemed to be a risk factor in women and sTNF-R2 might be a mediator in the risk relationship between overweight and diabetes with pancreatic cancer. In order to clarify the role of pro-inflammatory proteins and cytokines in the pathogenesis of exocrine pancreatic cancer, more prospective studies in large settings are needed, controlling for the potential bias of other conditions and stratifying by sex.

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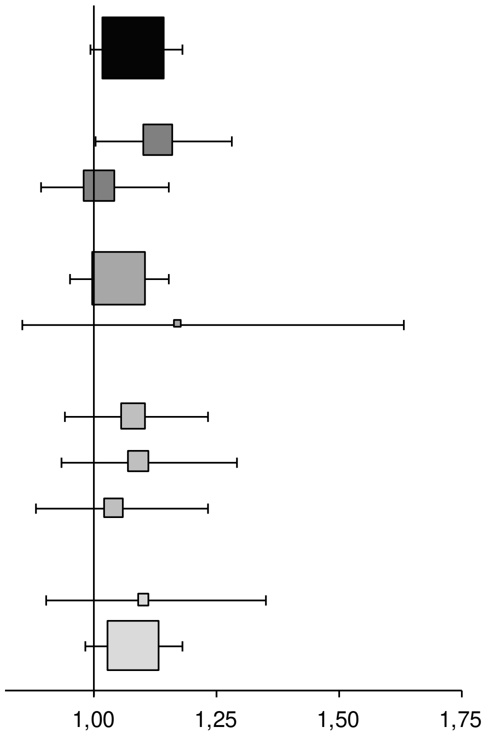
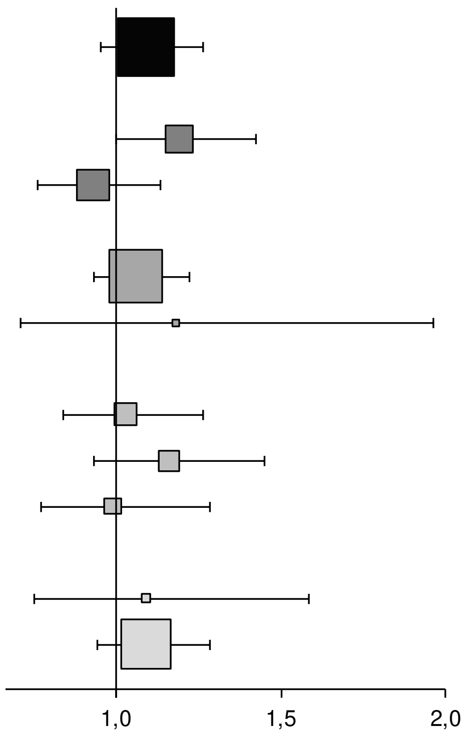
1c) CRP

Subgroup	Ca / Co	OR (95% CI)	P int [a]
All	449 / 449	1.08 (0.99-1.18)	
BMI < median [b]	200 / 226	1.13 (1.00-1.28)	0.3
BMI ≥ median	250 / 226	1.01 (0.89-1.15)	
Non-diabetics	373 / 401	1.05 (0.95-1.15)	0.1
Diabetics [c]	59 / 34	1.17 (0.85-1.63)	
Never smoker	161 / 197	1.08 (0.94-1.23)	0.9
Former smoker	143 / 149	1.09 (0.93-1.29)	
Current smoker	141 / 101	1.04 (0.88-1.23)	
FUP ≤ 2yrs [d]	77 / 77	1.10 (0.90-1.35)	0.9
FUP > 2yrs	373 / 375	1.08 (0.98-1.18)	



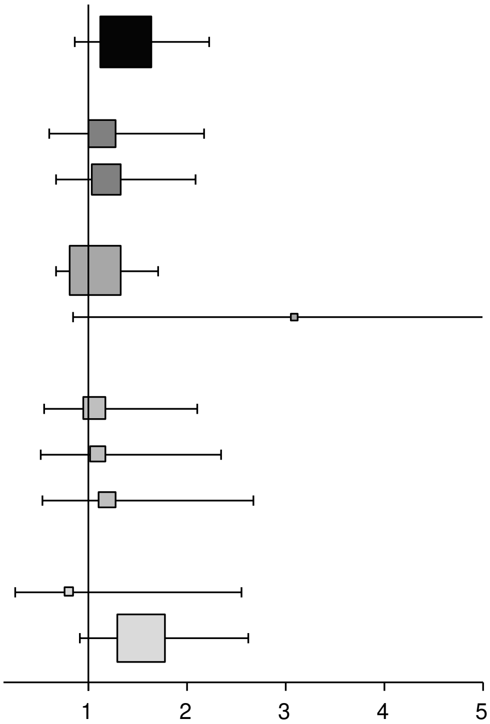
1d) IL-6

Subgroup	Ca / Co	OR (95% CI)	P int [a]
All	424 / 424	1.09 (0.95-1.26)	
BMI < median [b]	193 / 216	1.19 (1.00-1.42)	0.4
BMI ≥ median	247 / 221	0.93 (0.76-1.13)	
Non-diabetics	364 / 388	1.06 (0.93-1.22)	0.02
Diabetics [c]	59 / 33	1.18 (0.71-1.96)	
Never smoker	155 / 193	1.03 (0.84-1.26)	0.2
Former smoker	144 / 142	1.16 (0.93-1.45)	
Current smoker	136 / 97	0.99 (0.77-1.28)	
FUP ≤ 2yrs [d]	74 / 74	1.09 (0.75-1.58)	0.9
FUP > 2yrs	366 / 363	1.09 (0.94-1.28)	



1a) sTNF-R1

Subgroup	Ca / Co	OR (95% CI)	P int [a]
All	390 / 390	1.39 (0.87-2.23)	
BMI < median [b]	191 / 209	1.14 (0.60-2.18)	0.3
BMI ≥ median	236 / 203	1.19 (0.68-2.08)	
Non-diabetics	354 / 366	1.08 (0.68-1.70)	0.003
Diabetics [c]	56 / 30	3.09 (0.84-11.36)	
Never smoker	155 / 184	1.07 (0.55-2.11)	0.05
Former smoker	133 / 131	1.10 (0.52-2.34)	
Current smoker	134 / 92	1.20 (0.54-2.68)	
FUP ≤ 2yrs [d]	73 / 69	0.81 (0.26-2.55)	0.3
FUP > 2yrs	354 / 343	1.55 (0.92-2.62)	



1b) sTNF-R2

Subgroup	Ca / Co	OR (95% CI)	P int [a]
All	414 / 414	1.55 (0.99-2.44)	
BMI < median [b]	179 / 204	0.89 (0.50-1.58)	0.04
BMI ≥ median	238 / 213	1.93 (1.11-3.37)	
Non-diabetics	349 / 375	1.12 (0.73-1.72)	0.001
Diabetics [c]	54 / 29	4.76 (1.11-20.37)	
Never smoker	148 / 187	0.92 (0.47-1.81)	0.001
Former smoker	133 / 136	1.40 (0.69-2.84)	
Current smoker	131 / 89	1.61 (0.77-3.37)	
FUP ≤ 2yrs [d]	73 / 73	0.97 (0.34-2.80)	0.3
FUP > 2yrs	344 / 344	1.72 (1.05-2.84)	

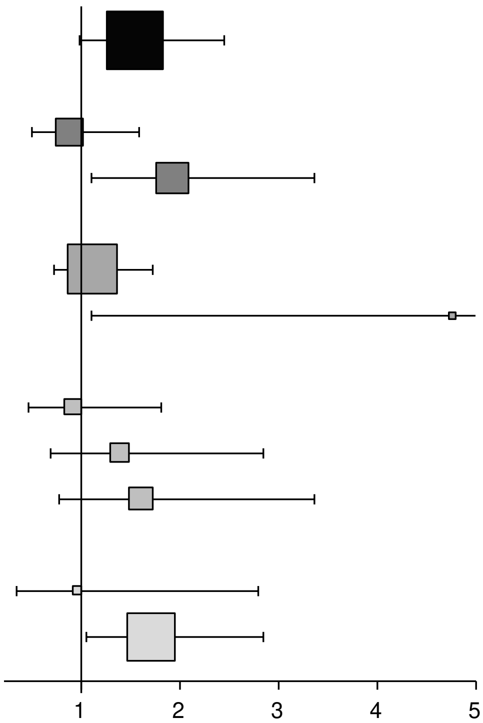


Table 1. Baseline characteristics of pancreatic cancer cases and matched controls

Variable	Cases (n=455)	Controls (n=455)	p-value[a]
Women, n (%)	235 (52)	235 (52)	matched
Age at recruitment [y], mean (range)	58 (30 – 76)	58 (30 – 76)	matched
Age at diagnosis [y], mean (range)	63 (37 – 82)	-	
Follow-up [y], mean (range)	5.3 (0 – 13)	-	
BMI [kg/m^2], mean ± SD			
Male	26.8 ± 3.6	26.7 ± 3.7	0.7
Female	26.5 ± 5.0	25.2 ± 4.3	0.002
Waist-hip ratio, mean ± SD			
Male	0.95 ± 0.06	0.95 ± 0.06	0.6
Female	0.82 ± 0.07	0.81 ± 0.06	0.09
Waist circumference [cm], mean ± SD			
Male	96.3± 9.9	96.7 ± 10.2	0.7
Female	84.4 ± 12.5	81.2 ± 10.7	0.001
Smoking status, n (%)			< 0.001
Never	162 (36)	198 (44)	
Former	145 (32)	151 (33)	
Current	143 (31)	101 (22)	
Unknown	5 (1)	5 (1)	
Alcohol intake at recruitment [g/d], mean ± SD			0.9
Male	21 ± 26	23 ± 31	
Female	9 ± 13	8 ± 11	
Fasting status, n (%)			matched
Fasting (≥ 6 hours)	118 (26)	113 (25)	
In between (3 - 6 hours)	78 (17)	78 (17)	
Non fasting (< 3 hours)	177 (39)	183 (40)	
Unknown	82 (18)	81 (18)	
Diabetes status, n (%)			
Self-reported diabetes at recruitment	33 (8)	19 (4)	0.05
Subjects HbA1c ≥ 6.5%	54 (12)	29 (6)	0.006
Self-reported diabetes or HbA1c ≥ 6.5%	59 (14)	34 (8)	0.01
Unknown	18 (4)	17 (4)	
CRP [mg/L], geometric mean (95% CI)			
Men	1.12 (0.97-1.29)	1.08 (0.94-1.25)	0.8
Women	1.24 (1.08-1.42)	0.97 (0.84-1.12)	0.02
IL-6 [pg/mL], geometric mean (95% CI)			
Men	1.79 (1.63-1.96)	1.69 (1.52-1.89)	0.6
Women	1.58 (1.43-1.74)	1.44 (1.31-1.59)	0.3
sTNF-R1 [ng/mL], geometric mean (95% CI)			
Men	1.33 (1.28-1.37)	1.36 (1.32-1.41)	0.3
Women	1.39 (1.34-1.44)	1.32 (1.28-1.36)	0.003
sTNF-R2 [ng/mL], geometric mean (95% CI)			
Men	2.31 (2.23-2.40)	2.28 (2.20-2.37)	0.5
Women	2.43 (2.35-2.51)	2.33 (2.26-2.40)	0.04

[a] *P* values for continuous variables were based on paired t tests; p values for categorical variables were based on generalized McNemar's tests

SD = standard deviation, CI = confidence interval

Note: matching factors were EPIC recruitment centre, sex, age at blood collection, date of blood donation, time of blood donation, fasting status, and use of hormones (in women)

Table 2: Correlation (95% CI) between inflammatory markers and selected covariates in control participants [a]

Covariate	CRP	IL-6	sTNF-R1	sTNF-R2
IL-6	0.44 (0.35 to 0.53)			
sTNF-R1	0.29 (0.18 to 0.39)	0.33 (0.22 to 0.42)		
sTNF-R2	0.27 (0.16 to 0.37)	0.23 (0.12 to 0.33)	0.65 (0.58 to 0.71)	
BMI	0.40 (0.30 to 0.49)	0.29 (0.18 to 0.39)	0.17 (0.06 to 0.28)	0.05 (-0.06 to 0.16)
Waist	0.32 (0.22 to 0.42)	0.31 (0.20 to 0.41)	0.21 (0.10 to 0.31)	0.10 (-0.01 to 0.21)
WHR	0.23 (0.13 to 0.34)	0.25 (0.14 to 0.35)	0.16 (0.04 to 0.26)	0.09 (-0.03 to 0.20)
HbA1c	0.16 (0.05 to 0.27)	0.09 (-0.02 to 0.20)	0.10 (-0.01 to 0.21)	0.01 (-0.10 to 0.12)
Diabetes [b]	1.37 (1.08 - 1.74)	1.49 (1.06 - 2.11)	1.41 (0.44 - 4.55)	1.30 (0.42 - 4.02)
Smoking [c]	1.30 (1.10 - 1.54)	1.36 (1.06 - 1.74)	1.60 (0.72 - 3.58)	0.97 (0.43 - 2.16)
Sex [d]	1.07 (0.95 - 1.21)	1.21 (1.02 - 1.44)	1.79 (0.97 - 3.31)	0.90 (0.50 - 1.62)
Age [e]	0.13 (0.03 to 0.23)	0.18 (0.08 to 0.28)	0.30 (0.20 to 0.39)	0.32 (0.22 to 0.41)

CRP denotes C-reactive protein, IL-6 interleukin 6, sTNF-R1 and sTNF-R2 soluble tumour necrosis factor receptor 1 and 2, BMI body mass index, waist waist circumference, WHR waist-to-hip ratio, HbA1c glycated haemoglobin, age age at recruitment.

[a] For continuous covariates Spearman's partial rank correlation coefficients were applied. For categorical covariates we used logistic regression. Both methods were performed in controls and adjusted for age, sex and EPIC recruitment centre if not stated otherwise.

[b] Diabetic (HbA1c ≥ 6.5% or self-reported diabetes at baseline) vs. non-diabetic participants

[c] Current vs. never smokers

[d] Men vs. women, adjusted for age and EPIC recruitment centre.

[e] Adjusted for sex and EPIC recruitment centre.

Table 3. Risk [OR (95% CI)] of pancreatic cancer by quartiles of CRP, IL-6, and sTNF receptors, all subjects combined and stratified by sex [a]

		Quartiles [b]					
		1	**2**	**3**	**4**	***P* trend [c]**	**OR for doubling in concentration**
CRP	Quartile cut-offs [mg/L]	0.02 - 0.51	0.52 - 1.04	1.05 - 2.05	2.06 - 34.07		
	No. cases / controls (total 449 / 449)	88 / 112	112 / 112	130 / 113	119 / 112		
	Crude [d]	1.0	1.30 (0.88-1.94)	1.45 (1.00-2.10)	1.36 (0.92-2.01)	0.3	1.08 (0.99-1.18)
	Adjusted for smoking, BMI	1.0	1.25 (0.83-1.88)	1.20 (0.80-1.79)	1.02 (0.66-1.57)	0.6	1.01 (0.92-1.11)
	Men, crude	1.0	1.38 (0.76-2.52)	0.98 (0.56-1.70)	1.23 (0.68-2.21)	0.9	1.02 (0.90-1.15)
	Adjusted for smoking, BMI	1.0	1.39 (0.75-2.58)	0.93 (0.52-1.66)	1.09 (0.58-2.04)	0.7	1.00 (0.88-1.13)
	Women, crude	1.0	1.15 (0.67-1.97)	2.14 (1.27-3.59)	1.44 (0.85-2.47)	0.1	1.16 (1.02-1.31)
	Adjusted for smoking, BMI	1.0	1.19 (0.67-2.11)	1.65 (0.92-2.98)	0.99 (0.54-1.81)	0.6	1.02 (0.89-1.18)
IL-6	Quartile cut-offs [pg/ml]	0.16 - 0.94	0.95 - 1.57	1.58 - 2.65	2.66 - 9.66		
	No. cases / controls (total 424 / 424)	86 / 106	123 / 106	108 / 107	107 / 105		
	Crude [d]	1.0	1.45 (0.98-2.15)	1.28 (0.85-1.93)	1.30 (0.84-2.00)	0.6	1.09 (0.95-1.26)
	Adjusted for smoking, BMI	1.0	1.29 (0.86-1.94)	0.97 (0.62-1.51)	1.01 (0.64-1.61)	0.7	0.99 (0.85-1.16)
	Men, crude	1.0	2.02 (1.11-3.68)	1.73 (0.92-3.26)	1.36 (0.70-2.64)	0.9	1.07 (0.86-1.32)
	Adjusted for smoking, BMI	1.0	1.88 (1.00-3.51)	1.51 (0.75-3.04)	1.21 (0.60-2.45)	0.6	1.00 (0.80-1.25)
	Women, crude	1.0	1.10 (0.65-1.87)	1.01 (0.58-1.75)	1.29 (0.72-2.33)	0.4	1.12 (0.92-1.36)
	Adjusted for smoking, BMI	1.0	0.92 (0.52-1.62)	0.71 (0.38-1.33)	0.83 (0.43-1.60)	0.7	0.96 (0.77-1.19)
sTNF-R1	Quartile cut-offs [ng/ml]	0.75 - 1.13	1.14 - 1.31	1.32 - 1.58	1.59 - 2.95		
	No. cases / controls (total 390 / 390)	86 / 97	84 / 98	120 / 98	100 / 97		
	Crude [d]	1.0	0.97 (0.63-1.49)	1.41 (0.94-2.12)	1.23 (0.78-1.94)	0.2	1.39 (0.87-2.23)
	Adjusted for smoking, BMI	1.0	0.84 (0.54-1.32)	1.18 (0.77-1.82)	0.95 (0.58-1.55)	0.9	1.10 (0.66-1.81)
	Men, crude	1.0	0.72 (0.39-1.33)	0.81 (0.44-1.49)	0.71 (0.36-1.39)	0.4	0.67 (0.34-1.35)
	Adjusted for smoking, BMI	1.0	0.71 (0.38-1.35)	0.79 (0.42-1.49)	0.64 (0.31-1.29)	0.3	0.63 (0.30-1.32)
	Women, crude	1.0	1.23 (0.67-2.28)	2.25 (1.26-4.00)	1.97 (1.02-3.79)	0.02	2.74 (1.37-5.47)
	Adjusted for smoking, BMI	1.0	1.03 (0.53-1.99)	1.75 (0.93-3.27)	1.47 (0.72-3.02)	0.2	2.05 (0.97-4.34)
sTNF-R2	Quartile cut-offs [ng/ml]	0.83 - 1.95	1.96 - 2.31	2.32 - 2.68	2.69 - 4.82		
	No. cases / controls (total 414 / 414)	90 / 103	102 / 104	99 / 104	123 / 103		
	Crude [d]	1.0	1.17 (0.77-1.77)	1.18 (0.75-1.85)	1.52 (0.97-2.39)	0.07	1.55 (0.99-2.44)
	Adjusted for smoking, BMI	1.0	1.15 (0.74-1.77)	1.08 (0.68-1.72)	1.42 (0.89-2.27)	0.2	1.40 (0.88-2.23)
	Men, crude	1.0	1.06 (0.59-1.92)	0.98 (0.51-1.90)	1.20 (0.63-2.29)	0.6	1.24 (0.66-2.33)
	Adjusted for smoking, BMI	1.0	1.02 (0.55-1.88)	0.92 (0.46-1.81)	1.27 (0.65-2.46)	0.4	1.35 (0.69-2.61)
	Women, crude	1.0	1.28 (0.71-2.29)	1.40 (0.76-2.60)	1.92 (1.00-3.67)	0.05	1.95 (1.03-3.69)

	Quartiles [b]					OR for doubling
	1	2	3	4	*P* trend [c]	in concentration
Adjusted for smoking, BMI	1.0	1.22 (0.65-2.28)	1.17 (0.60-2.28)	1.72 (0.86-3.44)	0.1	1.60 (0.80-3.17)

CI = confidence interval, No. = number. CRP, IL-6, and sTNF receptor concentrations on continuous scales were log2 transformed. Smaller number of subjects due to missing laboratory values.

[a] Crude p-interaction over quartiles, for CRP = 0.03, IL-6 = 0.2, sTNF-R1 = 0.09, sTNF-R2 = 0.8. BMI and smoking adjusted p-interaction, for CRP = 0.03, IL-6 = 0.2, sTNF-R1 = 0.1, sTNF-R2 = 0.9

[b] Quartile cut points were based on the distribution of controls

[c] *P* trend test was based on median values of each quartile

[d] Logistic regression conditioned on matching factors (EPIC recruitment centre, sex, age at recruitment, date at entry in the cohort, time between blood sampling and last consumption of foods and drinks, hormone use). Adjusting variables in further model: smoking (former smokers adjusted for quitting smoking (< 10 or ≥ 10 years ago), current smokers adjusted for number of cigarettes (1-9, 10-19, or ≥ 20)), and BMI (continuous, [kg/m^2]).

Title and Captain of Figure 1

Figure 1: Crude relative risks [OR (95% CI)] of pancreatic cancer for a doubling in sTNF receptor concentrations, CRP, and IL-6, all and stratified by median BMI (26.2 for men, 24.6 for women), diabetes, smoking status, and length of follow-up (≤ 2 vs. > 2yrs)

Captain

NOTE: Stratified analysis using unconditional logistic regression were adjusted for matching factors (EPIC recruitment centre, sex, age at blood collection, date of blood donation, time of blood donation, fasting status, and use of hormones). Ca / Co = number of cases / controls. Size of squares is proportional to number of participants in the respective subgroup; squares represent ORs, with error bars indicating 95% CIs.

[a] P for interaction was based on the Wald statistics, adjusted for matching factors.

[b] Median BMI for male controls was 26.20 kg/m2, for female controls 24.61 kg/m2.

[c] Diabetics included subjects with self-reported diabetes status at baseline and subjects with glycated haemoglobin (HbA1c) levels ≥ 6.5% or both.

[d] FUP = follow-up time [years], using conditional logistic regression.